Docket No.: HO-P02188US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Goran Forsberg et al.

Application No.: 09/900,766 Confirmation No.: 7699

Filed: July 6, 2001 Art Unit: 1645

For: NOVEL ENGINEERED SUPERANTIGEN FOR HUMAN THERAPY Examiner: P. A. Duffy

DECLARATION UNDER 37 CFR §1.132

Dear Sir:

I, Gunnar Hedlund, Ph.D., do hereby declare as follows:

1. I am a citizen of Sweden, residing at Gullregnsvangen 131E, S-224 56 Lund, Sweden.

2. I am employee of the assignee of the above-referenced patent application ("Application"), and I am familiar with the contents of the Application.

3. Currently, I am a Senior Scientist at Active Biotech AB. I am skilled in the areas of molecular and cell biology, immunology, biochemistry and cancer therapies.

4. I understand that the Patent and Trademark Office Examiner in charge of examining the Application is alleging that the pending claims do not find sufficient enablement support in the filed application.

5. I submit the following statements and supporting data.

A. The Cavallin et al. Paper

I have read the Cavallin paper entitled "The Spectral and Thermodynamic Properties of Staphylococcal Enterotoxin A, E, and Variants Suggest That Structural Modifications Are Important to Control Their Function" (J. Biol. Chem., 275:1665-1672 (2000)). It is my understanding that one skilled in the art would understand that the Cavallin paper, including the statement quoted by the Examiner, refers only to spectral and thermodynamic properties of enterotoxins. One skilled in the art would not interpret Cavallin to teach or suggest that mutations in a chimeric SEE/SEA enterotoxin may not result in predictable functions of the enterotoxins. The paper is silent on this issue.

B. The Lower the EC_{50} Value, the Higher the Activity

I have read in the Office Action dated January 5, 2005, including page 6, wherein the Examiner states: "SEE and variants thereof have a markedly reduced ability as compared to SEA to induce T-cell proliferation (see Cavallin et al, page 1671, column 1; of record)." This statement is incorrect. The data referred to by the Examiner in Cavallin (Table 1) shows the EC_{50} value of SEE and variants thereof. One skilled in the art knows that the EC_{50} is the concentration giving 50% of maximal effect (*See, e.g.,* Antonsson et al., *J. Immunol.* 158:4246, 1997 (of record)). The *lower* the EC_{50} value, the *lower* the concentration of the substance that is required to produce a response – and, therefore, *the higher the activity* (i.e., the lower the EC_{50} value, the higher the activity). Therefore, to the extent that the data in Cavallin have any bearing on functional properties of Staphylococcal enterotoxins, it shows that SEE and variants thereof are more active at inducing T cell proliferation than is SEA. This is the opposite of the Examiner's contention.

C. Table 1 of the Application Shows a Range of SADCC Activity

I have also read on the top of page 7 in the Office Action, where the Examiner states that the specification does not reasonably enable the claimed combinations of mutations because Table 1, allegedly, shows that some combinations of mutations, such as SEA/E-75, "abolish the SADCC activity," and therefore the specification is not reasonably predictive of the claimed combinations.

This is not a correct interpretation of the data shown in the application, including that in Table 1. Rather, and in fact, Table 1 shows that 24 different combinations of mutations have varying amounts of SADCC activity. For example, mutant SEA/E-75 has 0.1% of the SADCC activity of SEA/E-18. The SADCC activity of SEA/E-18 is substantial, and therefore a variant having 0.1% of that activity still has notable SADCC activity. Indeed, the data shown in Table 1 are LD_{50} values, the dose at which 50% of target cells are killed by SADCC. Therefore, a mutant like SEA/E-75, having 0.1% of the SADCC of SEA/E-18, means that it takes a dose that is just 10 fold higher than SEA/E-18 to kill 50% of cells by SADCC. Since the measured killing effect is killing 50% of cells by SADCC, this value still indicates that the variant has notable SADCC activity. In certainly does not indicate "abolish[ed]" SADCC activity.

Therefore, I understand that the data in the application, for example Table 1, shows a range of SADCC activities that can be obtained by the variants of the present claims.

D. In Vivo and In Vitro Use and Data

I understand from the Office Action of January 5, 2005, that the Examiner is concerned about whether the claimed invention functions *in vitro* and *in vivo*. For example, on the bottom of page 7, the Examiner questioned whether the specification teaches the full claimed scope of antibodies, or a molecule-binding antibody active fragments, that are directed against a cancer-associated cell surface structure.

First, the specification amply describes and enables the complete invention using, for example, antibody fragments C215Fab and 5T4Fab that are directed to a cancer associated cell surface antigens (see, e.g., paragraph 103). The specification also describes the use of antibodies to, for example, cell surface structures associated with cancers including cancers of the lung, breast, colon, kidney, pancreas, ovary, stomach, cervix and prostate (see paragraph 102). Specific examples of tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and p155 (see, e.g., paragraph 103).

Moreover, as the attached data shows, many different antibodies can be conjugated with the Staphylococcal enterotoxin variants of the present invention, and that the conjugate can successfully be used *in vitro* and *in vivo* against cells expressing the antigen recognized by the antibody. From the application and as supported by the attached data, one skilled in the art would understand that the variants of the instant invention may be conjugated with any antibody that recognizes a specific cell surface antigen, such as a cancer-associated antigen, and the conjugate can be successfully used both *in vitro* and *in vivo* to bind to and to kill target cells expressing the cell surface antigen recognized by the antibody. The attached data teaches both *in vitro* and *in vivo* success.

For example, in the attached Table I / IN VIVO, the data shows the successful use of the claimed invention wherein Staphylococcal enterotoxin variants are conjugated with antibodies, or molecule-binging antibody fragments, to various cancer cell surface molecules. The table shows that the conjugates both bind to cells expressing the target cancer antigen and have a therapeutic and/or pharmaceutical effect by killing the target cells.

In the attached Table II / IN VITRO, the data shows that variants of the claimed invention may be conjugated to antibodies, or molecule-binding antibody fragments, and the resulting conjugate used to specifically bind to and kill (via SADCC) target cells expressing the appropriate tumor target antigen.

I understand that from the patent application, and as supported by the attached data, one skilled in the art can reasonable predict, and without undue effort, make effective pharmaceutical conjugates of variants of Staphylococcal enterotoxins within the full scope of the claims as currently pending in this application.

D. SCID Mice Examples

On the top of page 7, and on pages 8 and 9 of the Office Action, the Examiner expresses concern as to whether to claimed conjugates function *in vivo*. The attached data clearly shows that the conjugates of the claimed invention function effectively *in vivo*. As shown in the attached data entitled "Therapy of Human Tumors with ABR-217620 in Humanized SCID Mice," a typical variant conjugate of the claimed invention

(5T4FabSEA/E-120), was successfully used to treat non-small cell lung carcinoma (NSCLC) in humanized SCID mice.

I understand that this data shows that the claimed invention is enabled to the treatment of cancers *in vivo*.

6. I hereby declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 04 May 2005

Gunnar Hedlund

Gunnar Hedlund, Ph.D.

Table 1 / IN VIVO

Cancer	Cell Line	Drug / Antibody	Therapeutic Effect	Antigen (FACS)
Melanoma				
	B16.C215	C215FabSEAm9-Q-hIL2	+	+
		5T4FabSEAm9-Q-hIL2	-	-
		C215FabSEA	+	+
		C242FabSEA	-	-
	B16.5T4neo	5T4FabSEAm9-Q-hIL2	+	+
		C215FabSEAm9-Q-hIL2	-	-
	FM3			
		K305FabSEA/E11	+	+
		C215FabSEA/E11	-	n.t
		K305FabSEAm9	+	+
		C215FabSEAm9	-	-
		XG2A3Fab-SEAm9	-	n.t
		K745FabSEAm9	+	+
		5T4FabV13SEAm9	+	+
		CD19FabSEAm9	-	-
Lymphoma	Daudi			
		CD19FabSEAm9	+	+
		C215FabSEAm9	-	-
Lung	Calu-1			
		5T4FabV13SEAm9	+	+
		CD19FabSEAm9	-	n.t
		C242FabSEAm9	-	n.t
		5T4FabSEA/E-18	+	+
		5T4FabSEA/E-11	+	+
		K305FabSEA/E-11	-	n.t
Colon	Colo-205			
		5T4FabSEA/E-18	+	+
		C242FabSEA	+	+
		K305FabSEA/m9	-	-
		C215FabSEAm9	+	+
		C215FabSEA	+	+
		C215FabSEA(E-11)	+	+
		SEA	-	-
	LS174T			
		242Fab-SEA	-	n.t
		C215FabSEA	+	+

TABLE I

The therapeutic effort of tumour targeted versus non-targeted superantigen was tested in different murine models with different cell lines. B16 melanoma cells, transfected with the C215 human colorectal cancer antigen or the 5T4 human oncofetal antigen, were injected intravenously to the mice to form tumours in the lung. (To score the effects + shows a significant reduction in the number of lung tumours and – means no effect.) FM3 cells, Daudi cells, Calu-1 cells, Colo 205 cells and LS174T cells were injected intraperitoneally into SCID mice and gave rise to solid tumours in the abdomen. (To score the effects + shows a significant reduction in the tumour mass and – means no effect.) To verify whether the products bound the target cells, the expression of the antigen on the cell surface was analyzed by FACS. (+ shows expression of antigen; - means no expression of antigen; n.t. means not tested; SEAm9 is SEA D2127A; SEA/E-11 is SEA/E-bdeg D227A, under the nomenclature of Cavallin et al., 2000, J. Biol. Chem., 275, 1665–1672; SEA/E-18 is SEQ ID NO:3; SEA/E-120 is SEQ ID No:2; and SEA/E-36 is SEA/E-18 with D70R).

Table II / IN VITRO

Cancer	Cell line	Drug / Antibody	Cytotoxic Effect SADCC	Antigen (FACS)
Hu.colon ca	Colo 205	C215Fab-SEAm9	+	+
		SEAwt	-	
		5T4FabV13-SEAm9	+	+
		SEAwt	-	
		C242Fab-SEAm9	+	+
		CD19Fab-SEAm9	-	
		K745FabVI-SEAm9	+	-
		CD19Fab-SEAm9	-	-
	HT29	C215Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
		5T4Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
Hu.kidney.ca	Caki-2	C215Fab-SEAm9	+	-
		SEAwt	-	
		5T4VI8h(CHCL) Fab-SEA/E-120	+	+
		SEAwt	-	
Hu.cervix ca	ME 180	C215Fab-SEAm9	+	+
		SEAwt	-	
		5T4VI8h(CHCL) Fab-SEA/E-120	+	+
		SEAwt	-	
Hu.lung.ca	Calu-1	C215Fab-SEAm9	+	+
		7EIIFab-SEAm9	-	
		5T4FabV13-SEAm9	+	+
		SEAm9	-	
Hu.squamous lung ca	GLC-p02	C215Fab-SEAm9	+	+
		SEAm9	-	
		5T4Fab-SEAm9	+	+
		SEAm9	-	
Hu.NSCLC	U1752	C215Fab-SEAm9	+	+
		K305Fab-SEAm9	-	
		5T4(7mut)Fab-SEAm9	+	+
		K305Fab-SEAm9	-	
Hu.Epidermoid ca	A431	C215Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
		5T4Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
Hu. B-cell lymphoma	RJ225	CLBFabV3-SEAm9	+	+
		C215Fab-SEAwt	-	
Murine B-cell lymphoma	38C13	1D3Fab-SEA/E-36	+	+
		C215Fab-SEAwt	-	

TABLE II

The cytoxic effect of tumour targeted versus non-targeted superantigen was tested in vitro in a standard chromium release assay called Superantigen Antibody Dependent Cell-mediated Cytotoxicity (SADCC). Several different tumour cell lines were used as targets and human SEA reactive T-cells as effector cells. It was found that the tumour cells were only killed in a significant way by the T-cells if the superantigen construct bound to the tumour cells. To measure cytotoxicity, in the table the following scoring was made. + shows significant T-cell mediated tumour cell killing and - means no tumour cell killing. To verify whether the products bound the target cells, the expression of the antigen on the cell was analyzed by FACS. (+ shows expression of antigen; - means no expression of antigen; n.t. means not tested; SEAm9 is SEA D2127A; SEA/E-11 is SEA/E-bdeg D227A, under the nomenclature of Cavallin et al., 2000, J. Biol. Chem., 275, 1665–1672; SEA/E-18 is SEQ ID NO:3; SEA/E-120 is SEQ ID No:2; and SEA/E-36 is SEA/E-18 with D70R).

Therapy of human tumors with ABR-217620 in humanized SCID mice

Final Report of the Study

2 SUMMARY

Immunotherapy of ABR-217620 (5T4Fab-SEA/E-120) against human non small cell lung carcinoma (NSCLC) was investigated in SCID mice humanized with human peripheral blood mononuclear cells (PBM). Therapy was started on day 5 and given daily for 8 days. Intraperitoneal growing Calu-1 (NSCLC) cells, expressing the relevant 5T4 antigen, showed reduced tumor mass after treatment with intravenous injections of ABR-217620.

4 INTRODUCTION

The aim of this study was to investigate ABR-217620 in a humanized mouse model. In contrast to ABR-214936 which has been investigated in clinical trials, ABR-217620 is designed not to bind human antiSEA antibodies. ABR-217620 contains a synthetic superantigen fused to a tumor reactive monoclonal antibody fragment (5T4). The therapeutic effect of ABR-217620 against human NSCLC in vivo was investigated using humanized SCID mice (1). The mice, which lack T and B-lymphocytes, can be reconstituted with human lymphocytes (2, 3) and used for tumor therapy (1, 4, 5). The therapeutic effect has previously been shown to be dependent on targeting of the superantigen to the tumor tissue and only occurs in the presence of PBM (6). Therefore, we injected human cancer cells intraperitoneally into mice followed by intraperitoneally injections of human PBMs and intravenous injections with ABR-217620 to investigate the therapy.

5 MATERIALS AND METHODS

5.1 Reagents

RPMI 1640 with Ultraglutamin+ was from Bio Whittaker, UK and was supplemented with 10% Fetal Bovine Serum from Gibco, UK.

Recombinant ABR-217620 and ABR-214936 was obtained by expression in *E. coli* and purified to homogeneity (7, 8). Phosphate buffered saline (PBS) (ICN Biomedicals INC., US), or PBS without magnesium and calcium (Bio Whittaker, Europe) were used and when injected supplemented with 1% Balb/c mouse serum (in house).

5.2 Animals

Severe Combined Immunodeficient (SCID) mice were bred in the animal facilities at Active Biotech Research AB. They were housed under sterile conditions with food and water ad libitum. The mice were at 8-12 weeks old when the studies started. Animal Ethical Committee in Malmö-Lund approved the studies.

5.3 Cells

The human NSCLC line Calu-1 was cultured in RPMI 1640 + 10% FCS. The cells were prepared by detachment with trypsin EDTA 1:250 (Bio Whittaker, UK), washed with PBS and resuspended in PBS containing 1% Balb/c mouse serum. The cells were injected into the mice within one hour of preparation. Peripheral blood mononuclear cells (PBM) were obtained from blood donors at the University Hospital of Lund. The PBMs were isolated by density centrifugation over a Ficoll-Paque cushion (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

5.4 Therapy

SCID mice were injected intraperitoneally with 3 x 10^6 Calu-1 cells in 0.2 ml vehicle (PBS - 1% Balb/c mouse serum). 5 days later the mice were injected intraperitoneally with 1 x 10^6 PBMs in 0.2 ml vehicle. One to two hours later, all mice were injected intravenous with test substance in 0.2 ml vehicle or vehicle alone. Intravenous injections with test substances or vehicle were given daily for 7 additional days. After 50 - 60 days the mice were sacrified by carbon dioxide inhalation aspleyxia and the number of tumors and the tumor mass was determined.

6 RESULTS

Since Calu-1 tumors grown intraperitoneally in SCID mice is a well-defined model that has been used for investigation of immunotheraphy with ABR-214936, it was used for therapy studies with ABR-217620. Studies of the tumor growth kinetics and use of PBM were carried out by Lando et al (6). Their experiments showed there is a good therapeutic effect when the treatment starts on 5-days-old tumors. Following their findings, we used the same number of PBM cells as tumor cells. There was a reduction in tumor mass, but also a high degree of variation in tumor mass in the control group. Treatment with PBM alone was also associated with an antitumor effect. Therefore we decided to give 3 times lower dose of PBM cells compared to Calu-1 cells and use 8 daily injections of drug. Using this improved protocol there was a statistically significant reduction in tumor mass after treatment with 16 µg ABR-217620, but notably the reduction was less pronounced with 160 µg ABR-217620 (see figure 1). The tendency that high doses of ABR-217620 give a poorer tumor therapy can be explained by killing of effector cells instead of tumor cells. Even though there was a decrease in tumor mass, there was no reduction in number of tumors.

A reason for variations between experiments is that PBMs from different doners were used in each study. For instance PBMs from some donors have a strong anti-tumoregenic activity by themselves. Therefore we tried different ratios of effector cells and target cells, and found that an E/T ratio of 1/3 minimised this effect.

Studies with a human kidney cancer cell line, Caki-2, has also been carried out. Caki-2 cells were generally more sensitive to PBM-s than Calu-1 cells and were not further investigated. Using the therapy protocol described above did not result in any reduction in tumor mass (data not shown).

7 CONCLUSIONS

ABR-217620 has a therapeutic effect onNSCLCtumor growth in humanized SCID mice.

8 FIGURES

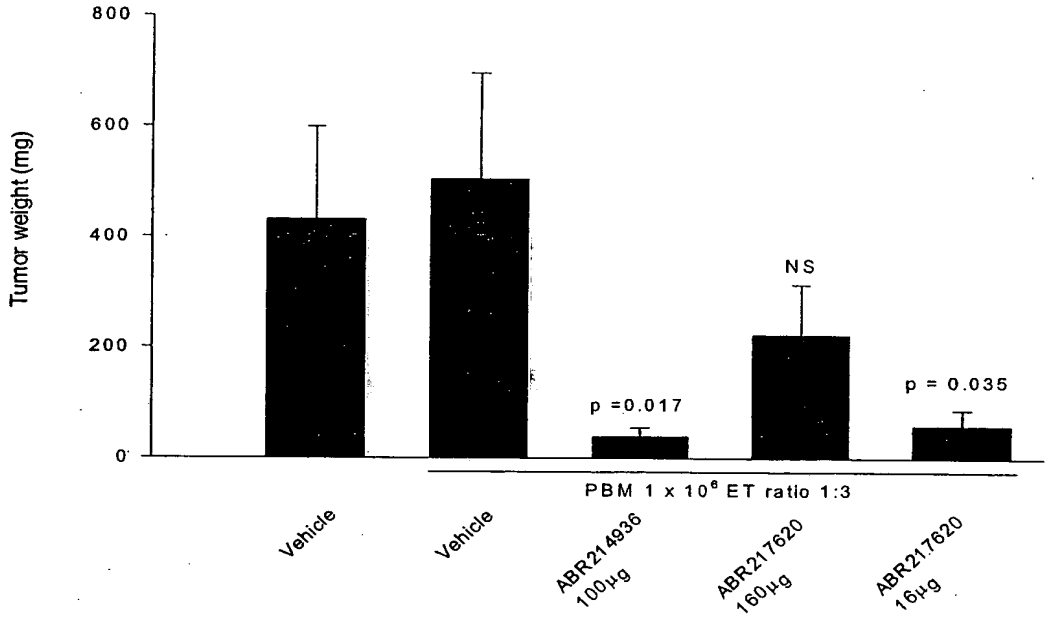

Figure 1. SCID mice with 5 day old intraperitoneally growing Calu-1 tumors were injected once intraperitoneally with PBM and 8 days intravenous with ABR217620. One experiment out of two.

9 REFERENCES

1. Lando, P.A., Dohlsten, M., Ohlsson, L. and Kalland, T. Tumor-reactive superantigens suppress tumor growth in humanized SCID mice. Int. J. Cancer, 62, 466-71,1995.

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8. Forsberg, G., Ohlsson, L., Brodin, T., Björk, P., Lando, P.A., Shaw, D., Stern, P.L. and Dohlsten, M. Therapy of human non-small-cell lung carcinoma using antibody targeting of a modified superantigen. Br. J. Cancer (2001) 85 (1): 129-136.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. ER264815255US0, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: May 5, 2005 Signature: Ronnie Webb (Ronnie Webb)

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Dear Sir:

I, Göran Forsberg, Ph.D., do hereby declare as follows:

1. I am a citizen of Sweden, residing at Sturegatan 52, 241 31 Eslöv Sweden

2. I am employee of the assignee of the above-referenced patent application ("Application"), I am a coinventor of the Application, and I am familiar with the contents of the Application.

3. Currently, I am Head of the Department of Scientific Affairs at Active Biotech AB. I am skilled in the areas of molecular and cell biology, immunology, biochemistry and cancer therapies.

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I have read the Office Action dated January 5, 2005, including, on page 5, the Examiner's quote from page 1671 of Cavallin that: "Interestingly, in many cases chimerical molecules of SEA and SEE acquire properties that are unique and not predicted combinations between SEA and SEE." I understand that the Examiner is using this quote to try to support the Examiner's argument that the claims of the patent application are not enabled by the specification. Specifically, the Examiner is using this quote to try to show that the art indicates that chimeric combinations of SEE and SEA have unexpected properties in the context of the claimed invention. As a coauthor of Cavallin paper, I disagree with this attempted characterization of this quote and of the disclosure of the Cavallin paper generally. The Cavallin paper and the quoted sentence is addressed to spectral and thermodynamic properties of Staphylococcal enterotoxins. For example, it studies and reports the effects of certain mutations in chimeric molecules on the spectral and thermodynamic of the molecule. The paper did not address therapeutic and/or pharmaceutical properties of the molecules , as do the claims of this patent application. The statement quoted by the patent Examiner only applies to the spectral and thermodynamic properties of enterotoxins. It is taken totally out of context in the Examiner's citation. This statement from Cavallin simply does not apply to the context of the present claims, which relate to pharmaceutical enterotoxin conjugates, such as pharmaceutical and/or anti-cancer agents.

In addition, it is my opinion that one skilled in the art would read the Cavallin paper, including the statement quoted by the Examiner, to refer only to spectral and thermodynamic properties of enterotoxins. One skilled in the art would not interpret Cavallin to teach or suggest that mutations in a chimeric SEE/SEA enterotoxin may result in unpredictable functions of the enterotoxins. The paper is silent on this issue.

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means that it takes a dose that is just 10 fold higher than SEA/E-18 to kill 50% of cells by SADCC. Since the measured killing effect is killing 50% of cells by SADCC, this value still indicates that the variant has notable SADCC activity. In certainly does not indicate "abolish[ed]" SADCC activity.

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bind to and to kill target cells expressing the cell surface antigen recognized by the antibody. The attached data teaches both *in vitro* and *in vivo* success.

For example, in the attached Table I / IN VIVO, the data shows the successful use of the claimed invention wherein Staphylococcal enterotoxin variants are conjugated with antibodies, or molecule-binging antibody fragments, to various cancer cell surface molecules. The table shows that the conjugates both bind to cells expressing the target cancer antigen and have a therapeutic and/or pharmaceutical effect by killing the target cells.

In the attached Table II / IN VITRO, the data shows that variants of the claimed invention may be conjugated to antibodies, or molecule-binding antibody fragments, and the resulting conjugate used to specifically bind to and kill (via SADCC) target cells expressing the appropriate tumor target antigen.

I understand that from the patent application, and as supported by the attached data, one skilled in the art can reasonable predict, and without undue effort, make effective pharmaceutical conjugates of variants of Staphylococcal enterotoxins within the full scope of the claims as currently pending in this application.

D. SCID Mice Examples

On the top of page 7, and on pages 8 and 9 of the Office Action, the Examiner expresses concern as to whether to claimed conjugates function *in vivo*. The attached data clearly shows that the conjugates of the claimed invention function effectively *in vivo*. As shown in the attached data entitled "Therapy of Human Tumors with ABR-217620 in Humanized SCID Mice," a typical variant conjugate of the claimed invention (5T4FabSEA/E-120, identical to SEQID 1), was successfully used to treat non-small cell lung carcinoma (NSCLC) in humanized SCID mice.

I understand that this data shows that the claimed invention is enabled to the treatment of cancers *in vivo*.

6. I hereby declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true; and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: May 4, 2005

Göran Forsberg, Ph.D.

Table 1 / IN VIVO

Cancer	Cell Line	Drug / Antibody	Therapeutic Effect	Antigen (FACS)
Melanoma				
	B16.C215	C215FabSEAm9-Q-hIL2	+	+
		5T4FabSEAm9-Q-hIL2	-	-
		C215FabSEA	+	+
		C242FabSEA	-	-
	B16.5T4neo	5T4FabSEAm9-Q-hIL2	+	+
		C215FabSEAm9-Q-hIL2	-	-
	FM3			
		K305FabSEA/E11	+	+
		C215FabSEA/E11	-	n.t
		K305FabSEAm9	+	+
		C215FabSEAm9	-	-
		XG2A3Fab-SEAm9	-	n.t
		K745FabSEAm9	+	+
		5T4FabV13SEAm9	+	+
		CD19FabSEAm9	-	-
Lymphoma	Daudi			
		CD19FabSEAm9	+	+
		C215FabSEAm9	-	-
Lung	Calu-1			
		5T4FabV13SEAm9	+	+
		CD19FabSEAm9	-	n.t
		C242FabSEAm9	-	n.t
		5T4FabSEA/E-18	+	+
		5T4FabSEA/E-11	+	+
		K305FabSEA/E-11	-	n.t
Colon	Colo-205			
		5T4FabSEA/E-18	+	+
		C242FabSEA	+	+
		K305FabSEA/m9	-	-
		C215FabSEAm9	+	+
		C215FabSEA	+	+
		C215FabSEA(E-11)	+	+
		SEA	-	-
	LS174T			
		242Fab-SEA	-	n.t
		C215FabSEA	+	+

TABLE I

The therapeutic effort of tumour targeted versus non-targeted superantigen was tested in different murine models with different cell lines. B16 melanoma cells, transfected with the C215 human colorectal cancer antigen or the 5T4 human oncofetal antigen, were injected intravenously to the mice to form tumours in the lung. (To score the effects + shows a significant reduction in the number of lung tumours and – means no effect.) FM3 cells, Daudi cells, Calu-1 cells, Colo 205 cells and LS174T cells were injected intraperitoneally into SCID mice and gave rise to solid tumours in the abdomen. (To score the effects + shows a significant reduction in the tumour mass and – means no effect.) To verify whether the products bound the target cells, the expression of the antigen on the cell surface was analyzed by FACS. (+ shows expression of antigen; - means no expression of antigen; n.t. means not tested; SEAm9 is SEA D2127A; SEA/E-11 is SEA/E-bdeg D227A, under the nomenclature of Cavallin et al., 2000, J. Biol. Chem., 275, 1665–1672; SEA/E-18 is SEQ ID NO:3; SEA/E-120 is SEQ ID No:2; and SEA/E-36 is SEA/E-18 with D70R).

Table II / IN VITRO

Cancer	Cell line	Drug / Antibody	Cytotoxic Effect SADCC	Antigen (FACS)
Hu.colon ca	Colo 205	C215Fab-SEAm9	+	+
		SEAwt	-	
		5T4FabV13-SEAm9	+	+
		SEAwt	-	
		C242Fab-SEAm9	+	+
		CD19Fab-SEAm9	-	
		K745FabVI-SEAm9	+	-
		CD19Fab-SEAm9	-	-
	HT29	C215Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
		5T4Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
Hu.kidney.ca	Caki-2	C215Fab-SEAm9	+	-
		SEAwt	-	
		5T4VI8h(CHCL) Fab-SEA/E-120	+	+
		SEAwt	-	
Hu.cervix ca	ME 180	C215Fab-SEAm9	+	+
		SEAwt	-	
		5T4VI8h(CHCL) Fab-SEA/E-120	+	+
		SEAwt	-	
Hu.lung.ca	Calu-1	C215Fab-SEAm9	+	+
		7EIIFab-SEAm9	-	
		5T4FabV13-SEAm9	+	+
		SEAm9	-	
Hu.squamous lung ca	GLC-p02	C215Fab-SEAm9	+	+
		SEAm9	-	
		5T4Fab-SEAm9	+	+
		SEAm9	-	
Hu.NSCLC	U1752	C215Fab-SEAm9	+	+
		K305Fab-SEAm9	-	
		5T4(7mut)Fab-SEAm9	+	+
		K305Fab-SEAm9	-	
Hu.Epidermoid ca	A431	C215Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
		5T4Fab-SEAm9	+	+
		K305Fab-SEA/E-11	-	
Hu. B-cell lymphoma	RJ225	CLBFabV3-SEAm9	+	+
		C215Fab-SEAwt	-	
Murine B-cell lymphoma	38C13	1D3Fab-SEA/E-36	+	+
		C215Fab-SEAwt	-	

TABLE II

The cytoxic effect of tumour targeted versus non-targeted superantigen was tested in vitro in a standard chromium release assay called Superantigen Antibody Dependent Cell-mediated Cytotoxicity (SADCC). Several different tumour cell lines were used as targets and human SEA reactive T-cells as effector cells. It was found that the tumour cells were only killed in a significant way by the T-cells if the superantigen construct bound to the tumour cells. To measure cytotoxicity, in the table the following scoring was made. + shows significant T-cell mediated tumour cell killing and - means no tumour cell killing. To verify whether the products bound the target cells, the expression of the antigen on the cell was analyzed by FACS. (+ shows expression of antigen; - means no expression of antigen; n.t. means not tested; SEAm9 is SEA D2127A; SEA/E-11 is SEA/E-bdeg D227A, under the nomenclature of Cavallin et al., 2000, J. Biol. Chem., 275, 1665–1672; SEA/E-18 is SEQ ID NO:3; SEA/E-120 is SEQ ID No:2; and SEA/E-36 is SEA/E-18 with D70R).

Therapy of human tumors with ABR-217620 in humanized SCID mice

Final Report of the Study

2 SUMMARY

Immunotherapy of ABR-217620 (5T4Fab-SEA/E-120) against human non small cell lung carcinoma (NSCLC) was investigated in SCID mice humanized with human peripheral blood mononuclear cells (PBM). Therapy was started on day 5 and given daily for 8 days. Intraperitoneal growing Calu-1 (NSCLC) cells, expressing the relevant 5T4 antigen, showed reduced tumor mass after treatment with intravenous injections of ABR-217620.

4 INTRODUCTION

The aim of this study was to investigate ABR-217620 in a humanized mouse model. In contrast to ABR-214936 which has been investigated in clinical trials, ABR-217620 is designed not to bind human antiSEA antibodies. ABR-217620 contains a synthetic superantigen fused to a tumor reactive monoclonal antibody fragment (5T4). The therapeutic effect of ABR-217620 against human NSCLC in vivo was investigated using humanized SCID mice (1). The mice, which lack T and B-lymphocytes, can be reconstituted with human lymphocytes (2, 3) and used for tumor therapy (1, 4, 5). The therapeutic effect has previously been shown to be dependent on targeting of the superantigen to the tumor tissue and only occurs in the presence of PBM (6). Therefore, we injected human cancer cells intraperitoneally into mice followed by intraperitoneally injections of human PBMs and intravenous injections with ABR-217620 to investigate the therapy.

5 MATERIALS AND METHODS

5.1 Reagents

RPMI 1640 with Ultraglutamin+ was from Bio Whittaker, UK and was supplemented with 10% Fetal Bovine Serum from Gibco, UK.

Recombinant ABR-217620 and ABR-214936 was obtained by expression in *E. coli* and purified to homogeneity (7, 8). Phosphate buffered saline (PBS) (ICN Biomedicals INC., US), or PBS without magnesium and calcium (Bio Whittaker, Europe) were used and when injected supplemented with 1% Balb/c mouse serum (in house).

5.2 Animals

Severe Combined Immunodeficient (SCID) mice were bred in the animal facilities at Active Biotech Research AB. They were housed under sterile conditions with food and water ad libitum. The mice were at 8-12 weeks old when the studies started. Animal Ethical Committee in Malmö-Lund approved the studies.

5.3 Cells

The human NSCLC line Calu-1 was cultured in RPMI 1640 + 10% FCS. The cells were prepared by detachment with trypsin EDTA 1:250 (Bio Whittaker, UK), washed with PBS and resuspended in PBS containing 1% Balb/c mouse serum. The cells were injected into the mice within one hour of preparation. Peripheral blood mononuclear cells (PBM) were obtained from blood donors at the University Hospital of Lund. The PBMs were isolated by density centrifugation over a Ficoll-Paque cushion (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

5.4 Therapy

SCID mice were injected intraperitoneally with 3 x 10^6 Calu-1 cells in 0.2 ml vehicle (PBS - 1% Balb/c mouse serum). 5 days later the mice were injected intraperitoneally with 1 x 10^6 PBMs in 0.2 ml vehicle. One to two hours later, all mice were injected intravenous with test substance in 0.2 ml vehicle or vehicle alone. Intravenous injections with test substances or vehicle were given daily for 7 additional days. After 50 - 60 days the mice were sacrified by carbon dioxide inhalation aspleyxia and the number of tumors and the tumor mass was determined.

6 RESULTS

Since Calu-1 tumors grown intraperitoneally in SCID mice is a well-defined model that has been used for investigation of immunotheraphy with ABR-214936, it was used for therapy studies with ABR-217620. Studies of the tumor growth kinetics and use of PBM were carried out by Lando et al (6). Their experiments showed there is a good therapeutic effect when the treatment starts on 5-days-old tumors. Following their findings, we used the same number of PBM cells as tumor cells. There was a reduction in tumor mass, but also a high degree of variation in tumor mass in the control group. Treatment with PBM alone was also associated with an antitumor effect. Therefore we decided to give 3 times lower dose of PBM cells compared to Calu-1 cells and use 8 daily injections of drug. Using this improved protocol there was a statistically significant reduction in tumor mass after treatment with 16 µg ABR-217620, but notably the reduction was less pronounced with 160 µg ABR-217620 (see figure 1). The tendency that high doses of ABR-217620 give a poorer tumor therapy can be explained by killing of effector cells instead of tumor cells. Even though there was a decrease in tumor mass, there was no reduction in number of tumors.

A reason for variations between experiments is that PBMs from different doners were used in each study. For instance PBMs from some donors have a strong anti-tumoregenic activity by themselves. Therefore we tried different ratios of effector cells and target cells, and found that an E/T ratio of 1/3 minimised this effect.

Studies with a human kidney cancer cell line, Caki-2, has also been carried out. Caki-2 cells were generally more sensitive to PBM-s than Calu-1 cells and were not further investigated. Using the therapy protocol described above did not result in any reduction in tumor mass (data not shown).

7 CONCLUSIONS

ABR-217620 has a therapeutic effect onNSCLCtumor growth in humanized SCID mice.

8 FIGURES

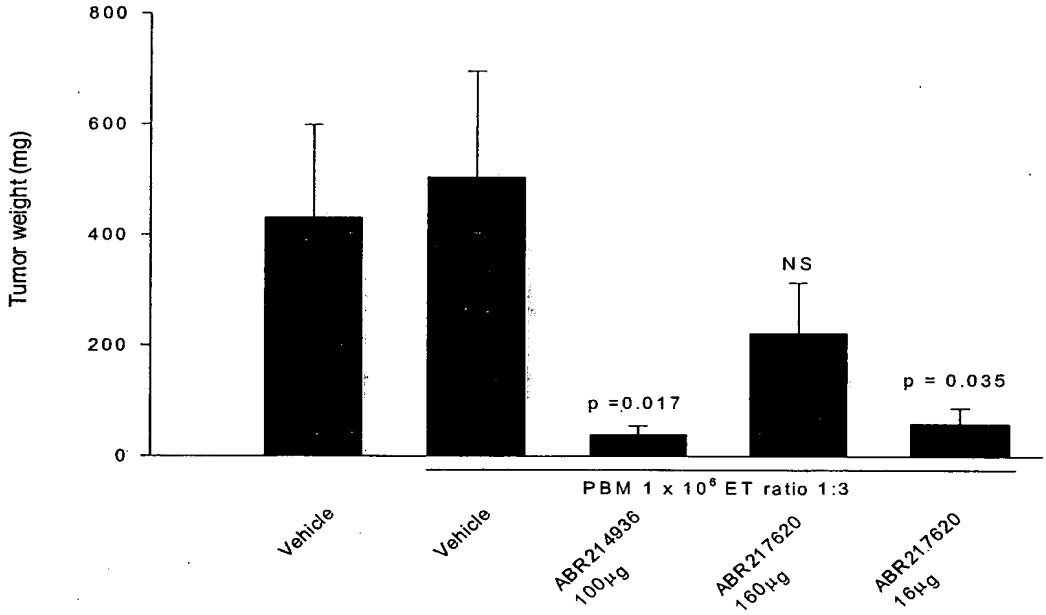

Figure 1. SCID mice with 5 day old intraperitoneally growing Calu-1 tumors were injected once intraperitoneally with PBM and 8 days intravenous with ABR217620. One experiment out of two.

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